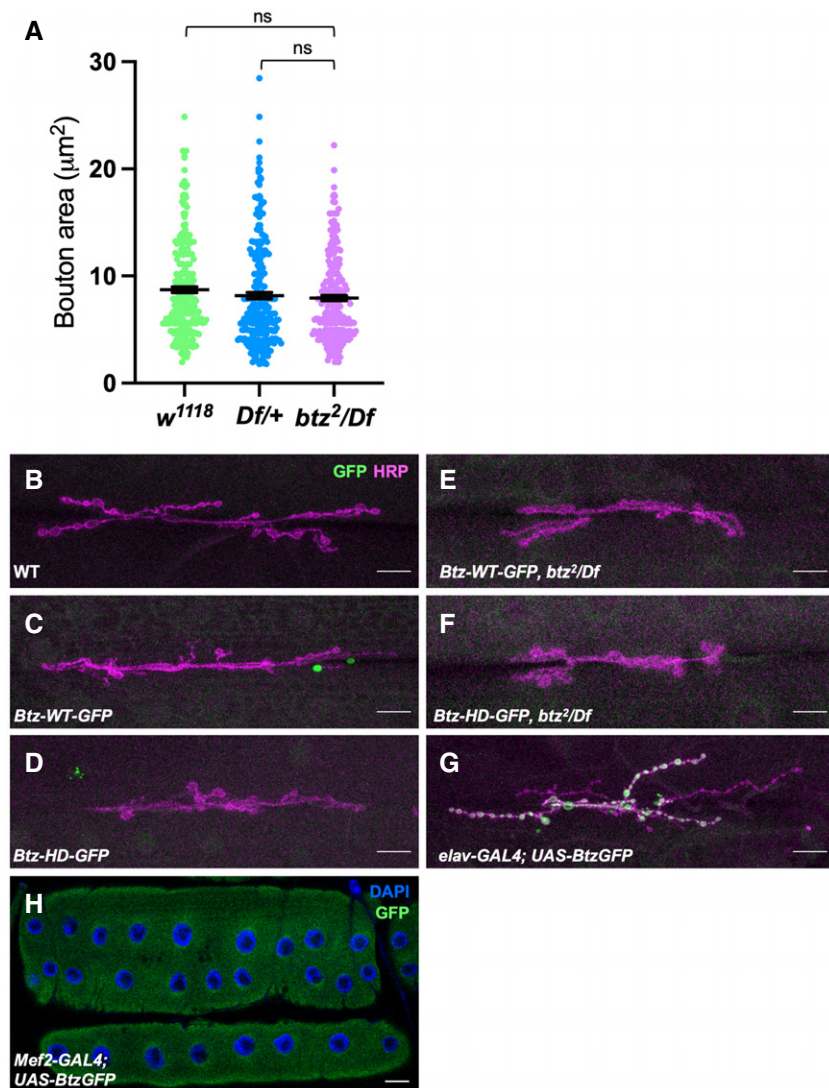


Expanded View Figures

**Figure EV1. Btz protein localization at the NMJ.**

A Quantification of the area of individual synaptic boutons at the NMJ on muscles 6/7 in segment A3 in *w¹¹¹⁸* and *Df(3R)BSC497/+* controls and in *btz²/Df(3R)BSC497* larvae. *btz* mutants show no significant reduction in bouton area compared to the controls. ns, not significant by Mann–Whitney test. $n = 248$ boutons from 6 NMJs (*w¹¹¹⁸*), 262 boutons from 6 NMJs (*Df/+*), or 246 boutons from 6 NMJs (*btz/Df*). Error bars show mean \pm SEM.

B–H Larval NMJs on muscle 6/7 in segment A3 stained with anti-HRP (magenta in B–G), DAPI (blue in H) and anti-GFP (green). (B) Canton S control; (C) *Btz-WT-GFP*; (D) *Btz-HD-GFP*; (E) *Btz-WT-GFP; btz²/Df(3R)BSC497*; (F) *Btz-HD-GFP; btz²/Df(3R)BSC497*; (G) *elav-GAL4; UAS-Btz-GFP*; (H) *Mef2-GAL4; UAS-Btz-GFP*. Tagged *Btz-WT* and *Btz-HD* expressed from the *btz* promoter fell below our level of detection, even in the absence of endogenous *btz*. GFP-tagged *Btz* overexpressed in neurons with *elav-GAL4* was localized to synaptic boutons, while GFP-tagged *Btz* overexpressed in muscle with *Mef2-GAL4* was distributed throughout the muscle. Scale bars, 30 μm .

Source data are available online for this figure.

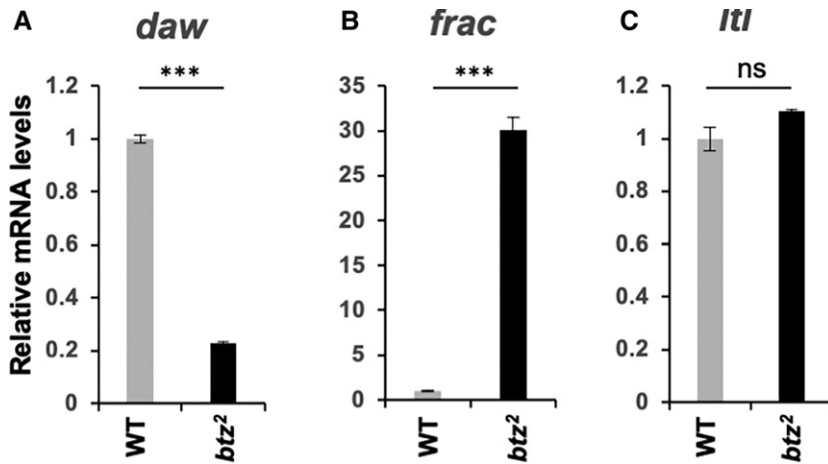


Figure EV2. Target gene expression in *btz* homozygotes.

A–C Relative mRNA levels measured by qRT–PCR for *daw* (A), *frac* (B), and *Itl* (C) in *btz*² homozygous larval carcasses compared to wild-type (Canton S). *daw* and *frac* show changes consistent with those seen in *btz*²/*Df*(3*R*)*BSC497*, but the change in *Itl* levels is not significant. ****p* < 0.001; ns, not significant by unpaired t-test. *n* = 3 for each sample. Error bars show mean ± SD.

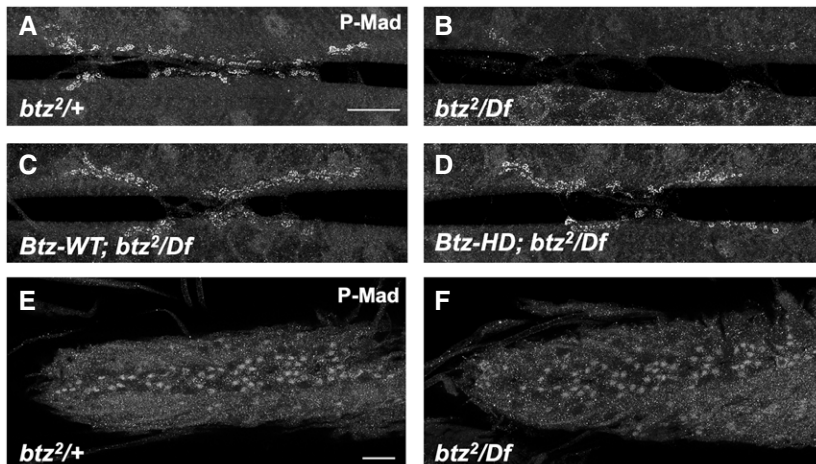


Figure EV3. *Btz* regulates P-Mad levels at the NMJ independently of the EJC.

A–F Confocal images of the NMJ on larval muscles 6 and 7 in segment A3 (A–D) or the larval ventral nerve cord (E, F), stained with anti-P-Mad. (A, E) *btz*²/*+* control; (B, F) *btz*²/*Df*(3*R*)*BSC497*; (C) *Btz*-WT; *btz*²/*Df*(3*R*)*BSC497*; (D) *Btz*-HD; *btz*²/*Df*(3*R*)*BSC497*. Scale bars, 20 μm. P-Mad is lost from the synapse but not from neuronal cell bodies in *btz* mutants, and is rescued by both the wild type and EJC interaction-defective *btz* transgenes.